Extended-Spectrum Beta-Lactamase-Producing Escherichia coli: A Global Threat to Antimicrobial Therapy and Infection Control

G. Soundhariya1 , A.Arjun1,a)

1Soundhariya Hospitals, Kollam, Kerala

**Corresponding Author :** a)[arjunsarkhar24@gmail.com](mailto:arjunsarkhar24@gmail.com)

**Abstract:** The global spread of ESBL producing bacteria, particularly Escherichia coli and Klebsiella pneumoniae, poses a critical threat to public health due to their multidrug-resistant nature. This study aimed to characterize an ESBL-producing E. coli strain by examining its biochemical properties, haemolytic activity, protein degradation potential, and β-Hexosaminidase activity. The isolated strain demonstrated typical biochemical characteristics of E. coli, including positive motility, indole production, and lactose fermentation, while showing negative results for urease and oxidase activities. Haemolytic assays revealed a concentration-dependent increase in red blood cell lysis, reaching 100% at 750 µg/ml and 1000 µg/ml. Similarly, protein degradation activity was observed to be concentration-dependent, with complete degradation occurring at 1000 µg/ml. Additionally, the strain exhibited significant β-Hexosaminidase activity, with 100% enzymatic activity recorded at the highest concentration tested. The study concludes that the strain’s haemolytic and enzymatic activities contribute to its pathogenicity, making it a challenging target for conventional antibiotic treatments.

**Keywords:** E. coli; ESBL; Haemolytic; Protein degradation; β-Hexosaminidase; Antibiotic

# Introduction

One of the most significant human pathogens that is known to cause a variety of extra-intestinal and intestinal diseases is Escherichia coli [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/abDXR6/tVhkl+Alrzu+xelPE)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/abDXR6/tVhkl+Alrzu+xelPE+TPlsv). AMR in E. Coli is observed globally, and rising E. Coli resistance rates are becoming a bigger issue in both developed and developing nations [(Wolde et al., 2024)](https://paperpile.com/c/abDXR6/vWesY). [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/abDXR6/ia6fi+xN4nB+4GRpf). The World Health Organization has classified ESBLs in Enterobacteriaceae as the primary cause of antimicrobial resistance (AMR), necessitating the development of novel medications [(Ullah et al., 2023)](https://paperpile.com/c/abDXR6/e1kMd). Escherichia coli can develop resistance to specific antibiotics even without prior exposure because it may acquire resistance genes from other bacteria that have already become resistant [(Uddin et al., 2021)](https://paperpile.com/c/abDXR6/2hCqH). Urinary tract infections (UTIs) are largely caused by Escherichia coli, a naturally occurring element of the human microbiome. E. Coli can transfer genetic material with other intestinal commensals, such as antibiotic resistance determinants, which can be plasmids or other mobile genetic components [(Dayie et al., 2024; Larramendy et al., 2020; Mahesh et al., 2024)](https://paperpile.com/c/abDXR6/ew6UV+PHYyS+tndAj). The presence of bacterial enzymes known as extended-spectrum β-lactamases (ESBLs) is often linked to resistance to other antimicrobial agents like aminoglycosides and fluoroquinolones [(Castanheira et al., 2021)](https://paperpile.com/c/abDXR6/Dzxw1). The genes encoding ESBLs are often located on plasmids, facilitating rapid horizontal gene transfer among bacterial populations [(Benz et al., 2021)](https://paperpile.com/c/abDXR6/avDie). There are several different kinds of ESBLs, but the TEM, CTX-M and SHV are the most common ones. The clinically relevant kinds PER, VEB, BES-1, SFO-1, BEL-1, IBC and TLA are among the other notable ones. Gram-negative bacteria belonging to the Enterobacteriaceae family, particularly Escherichia coli and Klebsiella pneumoniae, are the main producers of these enzymes [(Ribeiro et al., 2024; Saranya, 2020)](https://paperpile.com/c/abDXR6/uu8Be+DrgSI). E. coli and K. pneumoniae-containing extended-spectrum beta-lactamases (ESBLs) are common childhood infection causes and present serious problems, including treatment failure due to antibiotic resistance and high morbidity and mortality rates [(Sohail Afzal M, 2017; Sujatha et al., 2020)](https://paperpile.com/c/abDXR6/7ZmQS+gMHi4) [(Romyasamit et al., 2024)](https://paperpile.com/c/abDXR6/XMOG). Due to changes brought about by the extensive use of antibiotics in hospitals and animal farms, ESBL-producing bacteria have been able to adapt and proliferate [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/abDXR6/rW67+Rwqg+uFmu). Studies on the occurrence of ESBL-producing bacteria in inpatients have been shown, however they have primarily involved adult patients [(Kuralayanapalya et al., 2019; Romyasamit et al., 2024)](https://paperpile.com/c/abDXR6/paom+XMOG). The prevalence of ESBL-producing E. coli has increased dramatically, initially in hospital settings but now also in the community, driven by factors like international travel, globalization of food supply chains, and the overuse of antibiotics in both medicine and agriculture [(Husna et al., 2023)](https://paperpile.com/c/abDXR6/EzRr7). The spread of E. coli that produces ESBL-Ec, which undermines empirical treatment, exacerbates this issue [(Ling et al., 2024)](https://paperpile.com/c/abDXR6/dSye1) Antibiotic usage has led to an increased frequency of virulence and resistance profiles in ESBL-producing E. Coli from livestock, which has aided in the development of multidrug-resistant diseases [(Ramatla et al., 2024)](https://paperpile.com/c/abDXR6/lr3KV). The current study investigates ESBL producing E. coli, focusing on their protein degradation, anti-allergic activity, haemolytic activity, and biofilm activity. By examining these aspects, the study aims to gain a deeper understanding of the pathogenic mechanisms of ESBL-producing E. coli, which are known to cause a variety of challenging infections [( Pranati et al., 2021; Sakthi et al, 2021)](https://paperpile.com/c/abDXR6/KpnY+39FU+N09z)[(G. & Ganapathy, 2022; I. L. Kumar & Ramesh, 2021)](https://paperpile.com/c/abDXR6/B4lWM+ImEn0). The investigation into biofilm formation is particularly important, as biofilms can protect bacteria from antibiotic treatment, contributing to their persistence and resistance [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/abDXR6/wyOK+uWrL+Jk6M). Additionally, exploring the anti-allergic and haemolytic activities of these bacteria provides insights into their potential impact on host immune responses and the overall severity of infections they cause [(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/abDXR6/jdQ5+6Tjz+jBPt) [(Kuralayanapalya et al., 2019; Romyasamit et al., 2024)](https://paperpile.com/c/abDXR6/XMOG+paom).

# Materials and Methods

ESBL producing clinical isolates were obtained from and stored in airtight storage containers with proper label which has name of isolate, date of storage, name of culture medium and details of preservation solution(Saadh et al., 2024).Biochemical study was performed on the bacterial pathogen E. coli using samples obtained from the pathogen was streaked on MacConkey agar medium and its many biochemical characteristics were assessed using the criteria provided by Bergey's Manual ([Buchanan and Gibbons, 1974](https://doi.org/10.1111/j.1550-7408.1975.tb00935.x)) in order to identify it(Almatrafi et al., 2024). The triple sugar iron agar and other maltose test, the Voges-Proskauer test, the Methyl Red test, the lactose test, the catalase test, the citrate utilization test, the hydrogen sulfide (H2S) production test, the urease test, the inositol test, the sucrose test, the xylose test, and the starch test were among the tests used to measure the potential for producing indole. Red blood cells were obtained After resuspending the cells in three volumes of 1X PBS, they were centrifuged at 1000× g for 10 minutes at 4°C to wash them. After discarding the supernatant, the washing procedure was carried out twice more. Following the last washing, the sedimented cells were once again suspended in an equivalent volume of 1X PBS and kept cold until they were needed. To obtain final concentrations of 250, 500, 750, and 1000 µg/mL for the hemolytic activity assay, aliquots of the peptide solution were added to 100 µL of 1% rabbit erythrocytes in 1X PBS, which were enclosed in 2 mL centrifuge tubes. For one hour, the cell suspensions were incubated at 37°C. After being incubated, the non-lysed erythrocytes were extracted using a 16,000× g centrifugation method. Thermo Fisher, Waltham, MA, USA, used a Nanodrop One Spectrophotometer to detect the amount of released hemoglobin in the supernatant spectrophotometrically at 414 nm. In order to simulate no hemoglobin release, 1% erythrocytes were cultured in 1X PBS without peptide. 1% erythrocytes were cultured in 1X PBS containing 1% Triton X-100 as a positive control for 100% hemolysis [(Brunetti et al., 2020)](https://paperpile.com/c/abDXR6/sSTzw).The anti-inflammatory activity was assessed through a lipoxygenase inhibition assay using linoleic acid as the substrate. A 10 µL sample of alkaloid (at a concentration of 50 mg/mL) was mixed with 90 µL of lipoxygenase enzyme (400 U/mL) and incubated for 5 minutes at 25°C in darkness. Subsequently, 100 µL of 0.4 mM linoleic acid was added to each well, and the reaction mixture was further incubated for 20 minutes at 25°C in darkness. After this incubation, 100 µL of freshly prepared ferrous orange xylenol (FOX), consisting of 100 µM xylenol, 10 µM ferrous sulfate, and 30 µM sulfuric acid, was added. The mixture was incubated at 25°C for an additional 30 minutes. Following incubation, the optical density of the reaction mixture was measured at 560 nm using a UV-visible spectrophotometer (Almuhayawi et al., 2021). Additionally, cyclooxygenase-2 (COX-2) activity was evaluated using a COX assay kit (Sigma-Aldrich, USA).The β-hexosaminidase inhibitory activity was measured in accordance with the methodology previously detailed by [(Abd Rani et al., 2019)](https://paperpile.com/c/abDXR6/JWBf6). 2 × 105 E. coli cells were planted in 400 µl of medium per well of 24-well plates, and the plates were cultured overnight until 80% confluency was obtained. The cells were resuspended in 320 µl after being twice rinsed with 500 µL of enriched media. Following a 10-minute incubation period, 40 µl of diverse doses of actinobacteria-derived flavonoids were added to the cells and incubated for an additional 10 minutes. 30 minutes were spent incubating 20 µl of the allergen DNP-BSA (10 mg/ml). The supernatant was collected, and then it was centrifuged for 15 minutes at 1500 rpm and 8°C in a microcentrifuge tube. Six-well plates were inoculated with E. coli at a concentration of 104 cells/ml. The bacteria were allowed to form a biofilm over a period of 24 hours. Following biofilm establishment, the wells were treated with ceftazidime, with exposure times ranging from 12 to 48 hours. After 48 hours of incubation, the biofilms were stained using propidium iodide and acridine orange to differentiate between live and dead cells, allowing for the visualization of the treatment's effects.

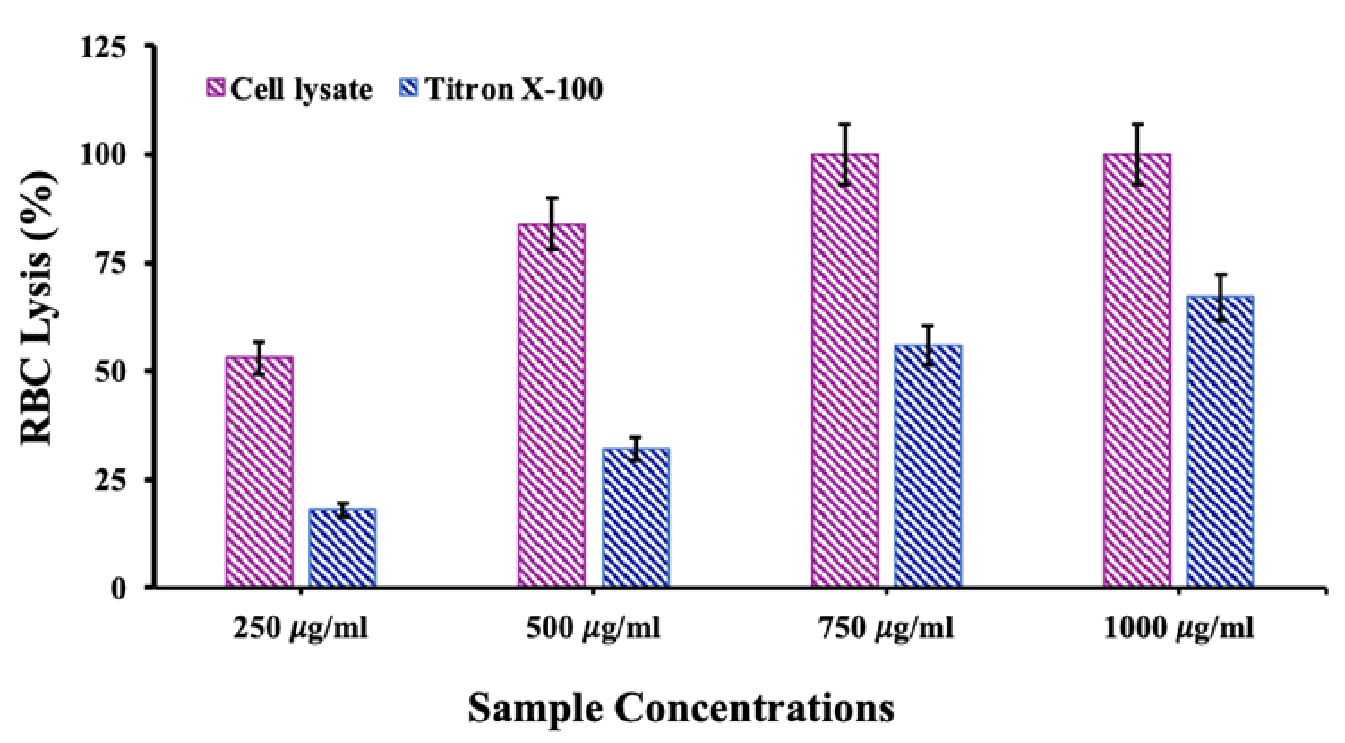
# Results

The Gram-negative, rod-shaped, bacterium Escherichia coli is a common member of the intestinal microbiota of warm-blooded animals and is widely studied for its clinical and environmental relevance. In this study, the isolate was confirmed as E. coli based on a comprehensive analysis of biochemical, carbohydrate fermentation, antibiotic susceptibility, and enzymatic tests (Table 1). The isolate was Gram-negative**,** rod-shaped**,** non-motile**,** non-spore formingand capsulated**,** with flagella but non motile and pigment production. In the basic biochemical tests, the isolate showed positive results in indole production, methyl red (MR), gas production, catalase activity, nitrate reduction**,** and oxidative/fermentative metabolism, suggesting strong metabolic flexibility and adaptation. It has shown negative result in Voges-Proskauer (VP), citrate utilization, oxidase, urease, hydrogen sulfide (H₂S) production, coagulase, gelatin liquefaction, niacin accumulation, bile solubility, Nagler’s reaction, and the string test. Hemolytic activity was alpha hemolysis, and the ONPG test was positive, confirming β-galactosidase activity indicates the lactose fermentation. Fermentation profiling exhibits that the isolate can utilize various carbon sources including arabinose, glucose, lactose, mannose, mannitol, sorbitol, starch, and xylose. Additionally, it cannot ferment fructose, inositol, maltose, ribose, and sucrose. This fermentation pattern of carbohydrates was typically highlighting the adaptive carbohydrate metabolism. Antibiotic efficacy of the isolate has shown resistance to bacitracin, novobiocin, optochin, and streptomycin**,** which indicates the efficiency of resistance mechanisms (Table 1).

**Table 1.** Biochemical identification of Escherichia coli

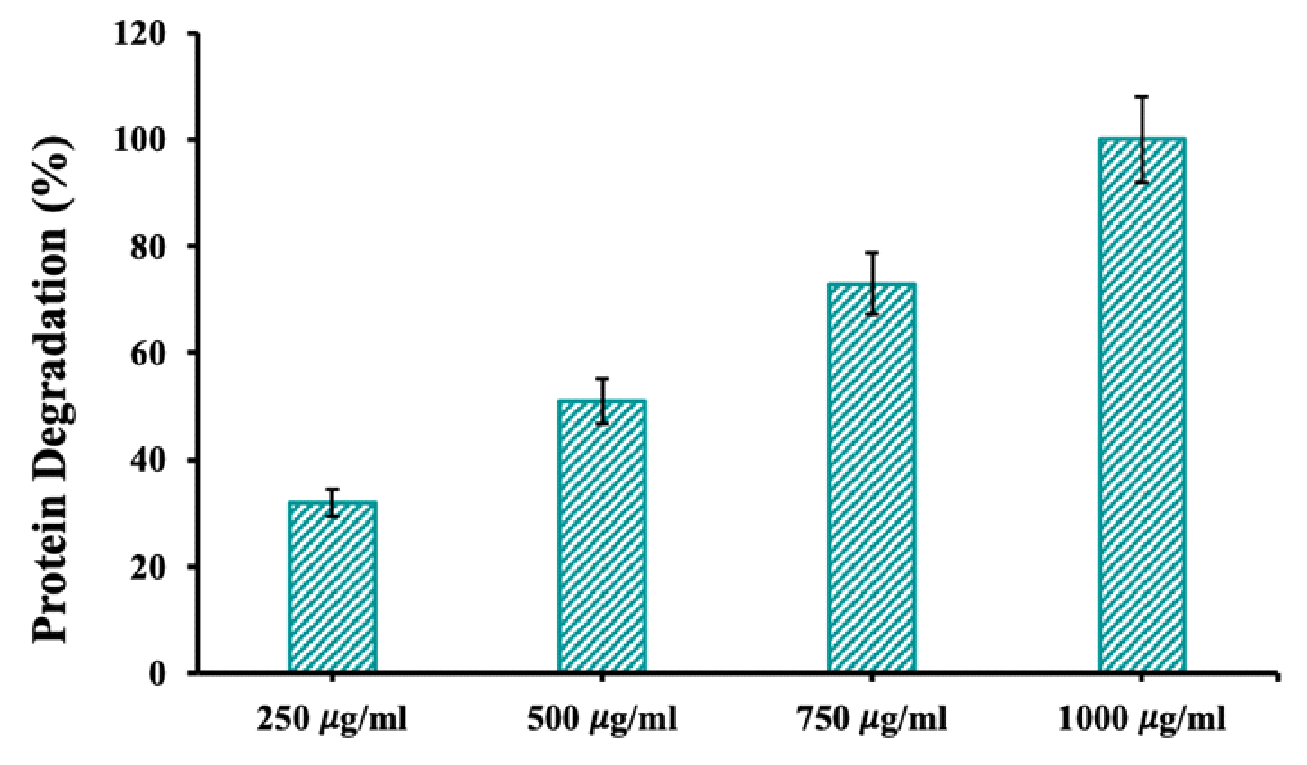
|  |  |
| --- | --- |
| **Biochemical Characteristics** | **Result** |
| Gram stain | Negative |
| Shape | Rod |
| Motility | Negative |
| Spore | Non sporing |
| Capsule | Capsulated |
| Flagella | Flagellated |
| Pigment | Negative |
| Indole | Positive |
| MR | Positive |
| VP | Negative |
| Citrate | Negative |
| Gas | Positive |
| Catalase | Positive |
| Oxidase | Negative |
| Urease | Negative |
| Nitrate Reduction | Positive |
| H2S | Negative |
| Oxidative/Fermentative | Positive |
| Coagulase | Negative |
| Hemolysis | Alpha |
| PYR | Negative |
| CAMP | Negative |
| Gelatin | Negative |
| Niacin | Negative |
| Bile Solubility | Negative |
| Nagler | Negative |
| Table 1 continued | |
| String Test | Negative |
| **Fermentation of carbon source** | |
| Arabinose | Positive |
| Fructose | Negative |
| Glucose | Positive |
| Inositol | Negative |
| Lactose | Positive |
| Maltose | Negative |
| Mannose | Positive |
| Mannitol | Positive |
| Ribose | Negative |
| Sorbitol | Positive |
| Starch | Positive |
| Sucrose | Negative |
| Xylose | Positive |
| **Antibiotic Efficacy** | |
| Bacitracin | Negative |
| Novobiocin | Negative |
| Optochin | Negative |
| Streptomysin | Negative |
| **Enzymatic reaction** | |
| DNase | Negative |
| Lipase | Negative |
| Amidase | Negative |
| Arginine dehydrolase | Positive |
| Perooxidase | Negative |
| Acid Phosphatase | Negative |
| Alkaline Phosphatase | Negative |
| Arylsulphatase | Negative |
| Beta Lactamase | Positive |
| Hyalurodinase | Negative |
| Lecithinase | Negative |
| Ornithine decarboxylase | Positive |
| Phenylalanine deaminase | Negative |
| Lysine | Positive |
| Acetate Utilization | Positive |
| ONPG Test | Positive |
| Tellurite | Negative |
| Tributyrin | Negative |
| Inference | E.coli |

In the enzymatic assay the isolate has shown positive results in arginine dehydrolase, β-lactamase, ornithine decarboxylase, and lysine decarboxylase, exhibits the ability of organisms to decarboxylate amino acids for energy production and stress response. Similarly, it has shown negative result for DNase, lipase, amidase, peroxidase, acid phosphatase, alkaline phosphatase, arylsulfatase, hyaluronidase, lecithinase, phenylalanine deaminase and acetate utilization-related enzymes, indicating the absence of many extracellular hydrolytic functions. Overall, the detailed biochemical and physiological characteristics confirm the isolate as Escherichia coli. Also, it indicates the adaptability of ESBL producing E.coli and survival in various environments associated with antibiotic resistance and biofilm formation.The haemolytic activity of the cell lysate was assessed at various concentrations (250, 500, 750, and 1000 µg/ml). At 250 µg/ml, the cell lysate induced 53% red blood cell lysis, with a standard deviation of 2.3. As the concentration increased to 500 µg/ml, RBC lysis rose to 84%, accompanied by a standard deviation of 2.7. At 750 and 1000 µg/ml concentrations, the cell lysate caused complete RBC lysis (100%), with standard deviations of 2.5 and 2.4, respectively. In comparison, Triton X-100, used as a positive control, exhibited increasing levels of haemolysis with concentrations corresponding to those of the cell lysate, resulting in 67% lysis at 1000 µg/ml (Fig. 1). These results indicate a concentration-dependent increase in haemolytic activity, with the cell lysate showing significantly higher lytic potential compared to Triton X-100 at equivalent concentrations.



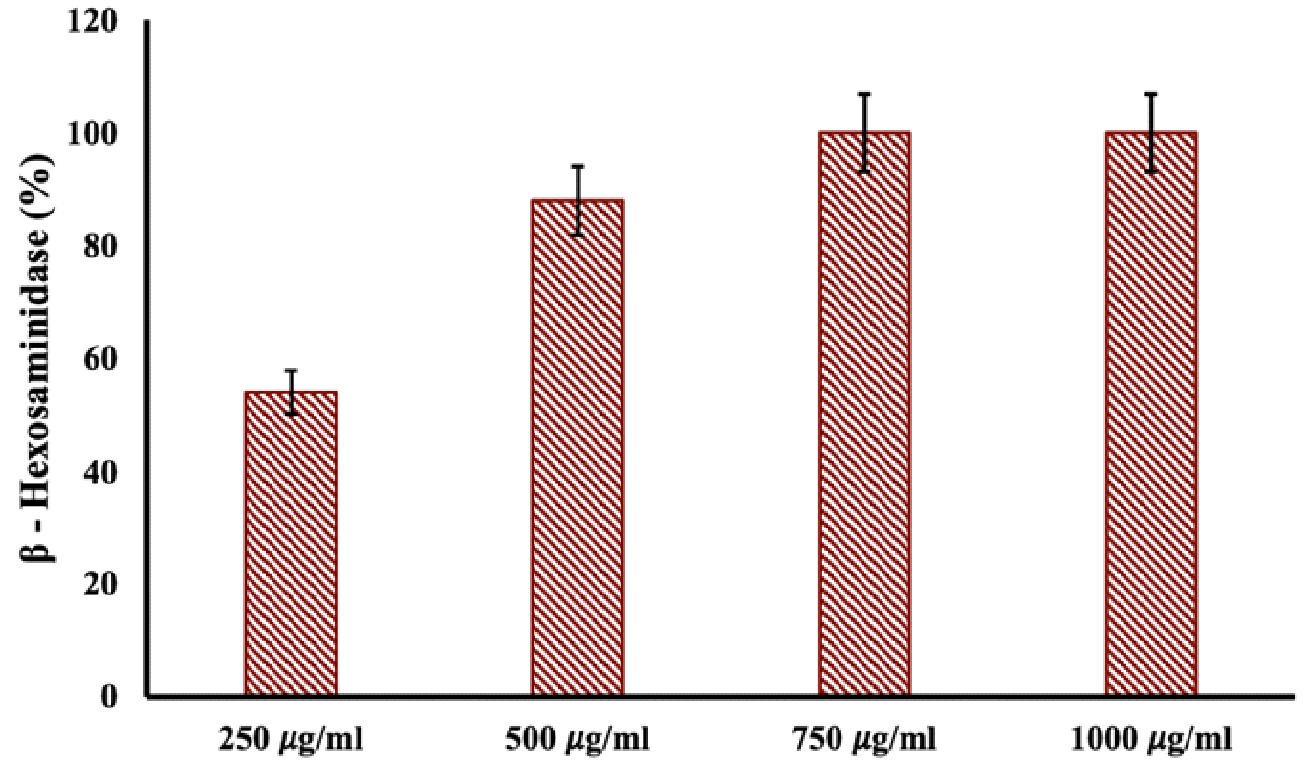
**Figure 1.** Haemolytic activity of beta lactamase (%) induced by cell lysate and Triton X-100 standard at varying sample concentrations (250–1000 µg/ml).

The increasing prevalence of multidrug-resistant E. coli strains that produce β-lactamases is a growing concern, as these enzymes exhibit diverse activities and resistance profiles among clinical isolates. The inflammatory activity associated with protein degradation was analysed at different concentrations of the cell lysate (250, 500, 750, and 1000 µg/ml). At the lowest concentration of 250 µg/ml, the cell lysate caused 32% protein degradation, with a standard deviation of 2.3. At the highest concentration tested, 1000 µg/ml, complete protein degradation (100%) was observed, with a standard deviation of 2.2 (Fig. 2). These results demonstrate a concentration-dependent increase in protein degradation linked to inflammatory activity, suggesting that higher concentrations of the cell lysate have a more significant impact on the degradation of inflammatory proteins.



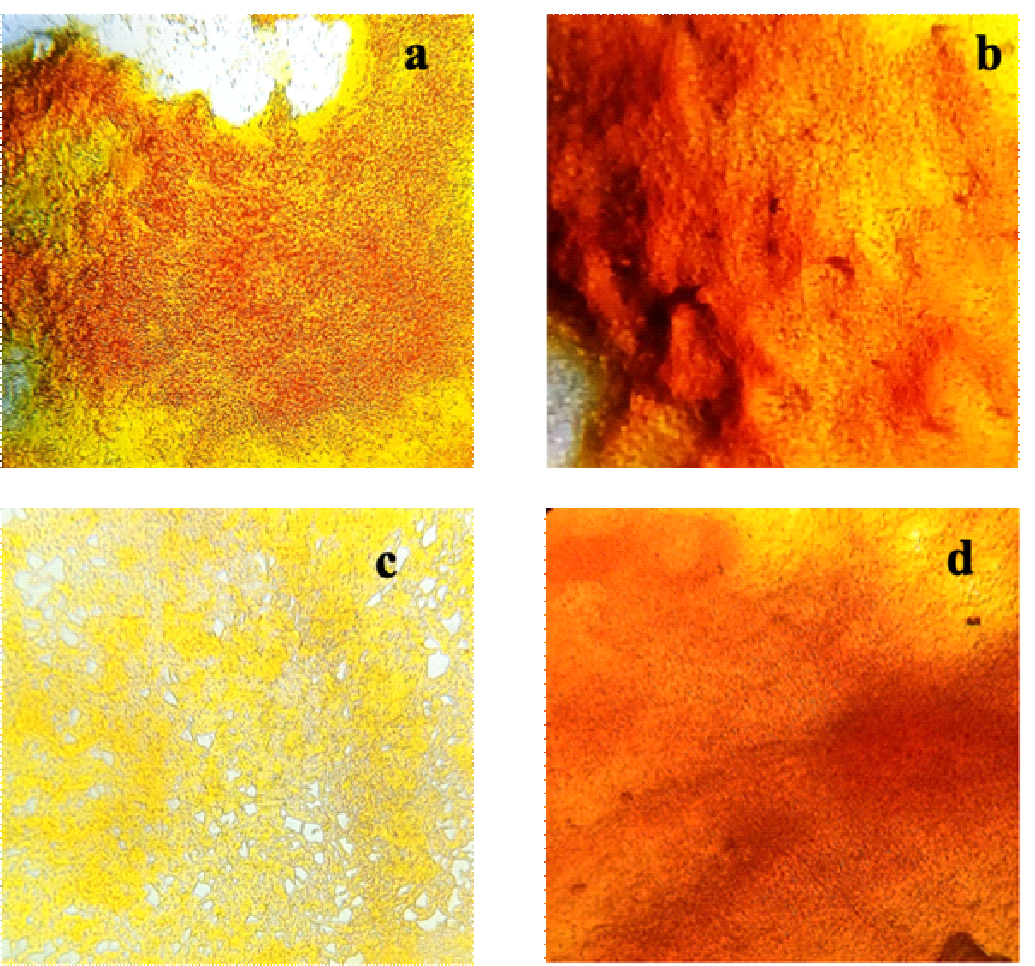
**Figure 2.** illustrates the protein degradation activity exhibited by β-lactamase, demonstrating its ability to break down target proteins, which reflects its enzymatic function and potential role in antibiotic resistance mechanisms.

The β-Hexosaminidase activity assay conducted using ESBL producing E. coli revealed significant enzymatic activity, indicating the presence of β-Hexosaminidase within these bacterial strains. This enzyme, responsible for the hydrolysis of glycosidic bonds in glycoproteins, glycolipids, and glycosaminoglycans, plays a critical role in bacterial metabolism and pathogenesis. The β-Hexosaminidase activity was evaluated at various concentrations of cell lysate (250, 500, 750, and 1000 µg/ml). At 250 µg/ml, the cell lysate exhibited 54% β-Hexosaminidase activity, with a standard deviation of 2.3. Increasing the concentration to 500 µg/ml resulted in an 88% activity level, with a standard deviation of 2.7. At 1000 µg/ml concentrations, the β-Hexosaminidase activity reached 100%, with standard deviations of 2.2 and 2.8, respectively (Fig. 3). Escherichia coli that produces ESBL is a cause for increasing concern worldwide because of its resistance to multiple drugs. Research has indicated a significant frequency of ESBL-producing E. Coli in clinical isolates, with a range of 21.1% to 58.82% [(Simanjuntak et al., 2023)](https://paperpile.com/c/abDXR6/Zg79E). These findings indicate a strong dose-dependent response, where higher concentrations of the cell lysate led to maximal β-Hexosaminidase activity. The findings offer novel prospects for investigating targeted therapeutic approaches and emphasizes the importance of the enzyme in comprehending the metabolic pathways of bacterial strains that are resistant to drugs.



**Figure 3.** shows the β-Hexosaminidase activity of the cell lysate, suggesting broader functional roles, including its ability to act on sugar-containing substrates.

The biofilm forming ESBL producing E.coli and their cell density was evaluated using acridine orange and propidium iodide staining at regular intervals. Initial observation at 12 hours (4a) showed initial adhesion and microcolony formation that has been improved significantly into mate formation at 24 hours (4b), the color intensity has shown the live cell density of ESBL E.coli in the biofilm matrix. The treated biofilm with 2:1 ratio amoxicillin-clavulanic acid for 12 hours (4c) has shown partial inhibition of ESBL producing E.coli forming Biofilm. At 48 hours (4d), a substantial reduction in both live and dead cells was distinct. It indicates that the antibiotic combination has significant efficacy in degrading the biofilm matrix and cell density. The results showed the ESBL producing E.coli has strongest biofilm formation which prevents the cells from targeted antibiotic therapy can significantly impair this process, highlighting its therapeutic potential in combating biofilm-associated infections.



**Figure 4.** Antibiofilm activity of E.coli a) biofilm forming ESBL producing E.coli a) 12 hours, b) 24 hours, The treated biofilm with 2:1 ratio amoxicillin-clavulanic acid c) 12 hours, d) 48 hours

# Discussion

The global proliferation of bacteria that produce ESBL, specifically Escherichia coli and Klebsiella pneumoniae, poses a significant challenge to the development of treatments for multidrug-resistant pathogens [(Chong et al., 2018)](https://paperpile.com/c/abDXR6/ywFDI).Haemolytic virulence factors are critical for bacterial pathogenicity, allowing them to flourish within host tissues, get energy from red blood cells, and elude the host's defensive mechanism [(K. B. V. Kumar et al., 2024)](https://paperpile.com/c/abDXR6/AQdhT). One of the main mechanisms of antibiotic resistance in Escherichia coli is the synthesis of beta-lactamases [(Winsou et al. 2023)](https://www.plantsjournal.com/archives/?year=2023&vol=11&issue=1&part=A&ArticleId=1512). Additionally, some strains of E. Coli generate hemolysins, which may enhance their pathogenicity. A hemolysin-producing E. Coli strain may also be more virulent [(Zoubi et al., 2020)](https://paperpile.com/c/abDXR6/BXuLG). 18.2% of STEC strains were discovered to carry the hlyA gene, which is linked to the synthesis of hemolysin, in a study of diarrheagenic E. coli in children. Multidrug-resistant germs were more prevalent at 24%, although strains that produced extended-spectrum β-lactamase (ESBL) were relatively low at 10% [(Mare et al., 2020)](https://paperpile.com/c/abDXR6/GPZgk). Under extreme circumstances, E. coli can produce highly active β-lactamases like NDM-35, co-produce other β-lactamases, and change proteins involved in antibiotic uptake in order to develop resistance to almost all β-lactams [(Poirel et al., 2022)](https://paperpile.com/c/abDXR6/UXp1h). According to the study, Xu et al., reported the innovative antimicrobial peptide enclosed in a cephalosporin showed reduced cytotoxicity and homolytic action, and it exhibited selective efficacy against bacteria that produce beta-lactamases [(Xu et al., 2023)](https://paperpile.com/c/abDXR6/GheYn)Protein degradation poses a major challenge in the production of recombinant proteins in Escherichia coli, especially for proteins that are secreted into the periplasmic space [(Bhatwa et al., 2021; Gawin et al., 2020)](https://paperpile.com/c/abDXR6/TTnNQ+15Sqk). β-lactam antibiotics can induce autolytic degradation of the cell wall in E. coli, with varying degrees of effectiveness based on their affinity for different penicillin-binding proteins [(Almeida et al., 2023; Vasconcelos et al., 2018)](https://paperpile.com/c/abDXR6/poaLm+sMNw6).

# Conclusion

This study emphasizes the multifaceted nature of Escherichia coli pathogenicity, particularly in strains producing extended-spectrum β-lactamases. The biochemical analysis confirms the presence of typical E. coli traits and reveals significant haemolytic, protein degradation, and β-Hexosaminidase activities. The observed concentration-dependent responses in haemolysis and protein degradation suggest that these factors may contribute significantly to the virulence and pathogenicity of the bacterium. Furthermore, the high β-Hexosaminidase activity in ESBL-producing E. coli indicates the enzyme's potential role in bacterial metabolism and resistance mechanisms. These results provide critical insights into the behavior of multidrug-resistant E. coli and offer new avenues for therapeutic research targeting these resistant strains. Addressing the global challenge posed by ESBL-producing bacteria will require a deeper understanding of their biochemical and pathogenic characteristics, as well as the development of novel strategies to mitigate their impact on public health.

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